Considered - RWK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Nicolaides *et al.*

Serial No.: 09/558,149 **Group Art Unit:** 1632

Filing Date: April 26, 2000 **Examiner:** R. Shukla

For: METHODS FOR GENERATING HYPERMUTABLE ORGANISMS

Considered JW 12/2/06

Assistant Commissioner for Patents
Washington D.C. 20231

Declaration under 37 CFR 1.131

I, J. Bradford Kline, hereby state the following:

1. I earned my doctorate from the University of Miami, Department of Microbiology and Immunology in 1996 in the field of Molecular Microbial Pathogenesis.

2. I am thoroughly familiar with the field of transgenic animals, and I am a Senior Scientist working on transgenic animals for Morphotek Inc. in the Mammalian Genetics Group.

3. I have been employed by Morphotek since May 2001, and have been working under the direction of Drs. Nicholas C. Nicolaides, Luigi Grasso and Philip M. Sass on transgenic animals expressing dominant negative alleles of mismatch repair genes.

4. I have read and understand the disclosure regarding the generation of transgenic animals in U.S. Patent No. 6,146,894 to Nicolaides *et al.* ("the ''894 patent").

5. Following the guidelines of the '894 patent, we generated transgenic mice as follows:

Transgene Fragment Generation

A plasmid containing a sequence for a PMS-134 truncation mutant driven by a pEF promoter was constructed and a fragment of the plasmid containing the promoter and PMS-134 sequence was restriction digested and purified by agarose gel electrophoresis and subsequent electroelution in TBE buffer. The fragment was ethanol precipitated, washed twice with

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Supplemental Declaration under 37 CFR 1.131

I, J. Bradford Kline, hereby state the following:

1. This Supplemental Declaration is provided to supplement my Declaration provided in the above-referenced case on September 26, 2002.

2. The transgenic mice that were generated comprising the PMS2-134 truncation mutant mismatch repair gene were examined for defects in mismatch repair resulting in hypermutability according to the following method of examining microsatellite instability (a hallmark of hypermutability):

Microsatellite Instability in the Morphomouse Genome

Wildtype or morphomouse genomic DNA was purified from the liver and diluted to 1pg/µl. 1 pg (0.5 haploid genome equivalents) was amplified by polymerase chain reaction (PCR) in a total volume of 12.5 µl using primers specific for the microsatellite marker mBat-37 using the following reaction conditions:

Touchdown cycle -
95 5min
94 1min 60 1min 72 1min
94 1min 59 1min 72 1min
94 1min 58 1min 72 1min
94 1min 57 1min 72 1min
94 1min 56 1min 72 1min
94 1min 55 1min 72 1min
94 1min 54 1min 72 1min